IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	)	Group Art Unit: 1645
McKENZIE et al.	)	Examiner: Zeman, R.
Serial No.: 09/163,089	)	
Filed: September 29, 1998	)	DECLARATION OF GEOFFREY A PIETERSZ (37 CFR 1.132)
Atty. File No.: 5036-1 (formerly 4102-1)	)	
For: COMPOSITIONS FOR IMMUNOTHERAPY AND USES THEREOF	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Geoffrey A. Pietersz, declare as follows:

1. I am a co-inventor of the above-referenced patent application and am familiar with the application. I am a skilled artisan in the field of immunology/chemistry.

2. This Declaration is being submitted in conjunction with an Amendment and Response to the Office Action having a mailing date of April 26, 2004.

3. The following discussion is provided in traverse of the Examiner's rejection of Claims 1, 3-17, 19-21, 24-26, 38 and 70-72 under 35 U.S.C. § 112, first paragraph.

4. This Declaration is provided as a Supplement to the Declaration under 37 CFR 1.132 previously submitted in the above-identified application on December 18, 2002, and provides more detail regarding the previously described experiments as they relate to the claimed invention. Specifically, the following data demonstrate the use of isolated mannose receptor-bearing cells and a conjugate comprising a tumor antigen (where the antigen is non-Muc1) and a carbohydrate polymer comprising mannose, wherein said carbohydrate polymer is a fully oxidized carbohydrate polymer comprising free aldehydes, to induce cellular immune responses in animals *in vivo*.

Dendritic Cells (the isolated mannose receptor-bearing cells)

H-2K^b C57BL/6 female 6-8 week old mice were used in the experiments. Mice were bred at the Austin Research Institute Biomedical Animal Research Lab

Bone marrow cells from C57BL/6 female mice were cultured at 10^6 cells/ml in tissue culture. Petri dishes contained RPMI 1640 medium (Gibco, NY, USA) supplemented with 1000 units/ml granulocyte and macrophage colony stimulating factor (GM-CSF), 10 μg/ml of interleukin-4 (IL-4), 10% heat inactivated fetal calf serum (FCS), 4mM L-glutamine, 100 U/ml penicillin, 100 :g/ml streptomycin sulphate and 100mM β-mercaptoethanol. At day 6 cells showed markers of mature dendritic cells (DCs), and expression of the mannose receptors

***Ex vivo* Treatment of Dendritic Cells with Oxidised Mannan-Antigen Conjugates to Produce the Composition Comprising Isolated Mannose Receptor-bearing Cells and a Conjugate Comprising a Tumor Antigen and a Carbohydrate Polymer Comprising Oxidised Mannose**

The mannose receptor-bearing dendritic cells as described above were washed, resuspended in the same culture media at $1x10^6$ cells/ml. A conjugate of oxidized mannan-Cripto, prepared as described for the oxidized mannan-MUC1 conjugates described in the present application (see Example 1), was loaded on to the DCs for 2 hours by adding the conjugate to the culture medium. This resulted in the generation of Cripto Pulsed DCs

Cripto is a protein expressed in embryonic and cancer cells. It is not expressed in normal tissues. The sequence of CRIPTO used here is a 17-mer peptide that is identical in both human and mouse CRIPTO. The sequence is: CPPSFYGRNCEHDVRKE, and is an antigenic portion of the Cripto protein.

Cripto Pulsed DCs were then washed thoroughly, resuspended at $1x10^7$cells/0.5ml in PBS (phosphate buffered saline) and 50 :l was injected intradermally in mice in the hind footpads. 10 days later mice were boosted. The pulsed DC's as described above meet the limitations of the composition of the invention as set forth in the present application on page 12, line 14 to page 13, line 4, because the pulsed DC's, even after washing, can include: (1) a mixture of conjugate and receptor-bearing cells wherein the conjugate is bound to the cells, but not yet internalized; (2) receptor-bearing cells wherein the conjugate has been internalized; (3)

receptor-bearing cells wherein the conjugate has been internalized and processed; and/or (4) receptor-bearing cells wherein the conjugate has been internalized, processed and presented.

Antigen Recall in Mice Treated with Pulsed Dendritic Cells

After 10-14 days, mice were sacrificed and splenocytes were isolated. Antigen recall (to measure the ability of the administered DCs to stimulate the immune response *in vivo* upon infection by an antigen) was assessed by ELISPOT IFN-gamma assays (which are a measure of T cell immune response) after addition of test or control antigens.

The test antigen was the Cripto 17-mer epitope described above. The control antigen was from an epitope comprising the Variable Number of Tandem Repeats (VNTR) of amino acid residues from Mucin1 (Muc1), a protein expressed on various tumour cells. In addition, a positive control consisting of ConA was used.

The results are shown in the attached figures.

As can be seen from the figures, producing a composition as claimed in the present application by pulsing DCs with a conjugate comprising oxidized mannan-Cripto in accordance with the invention described in the present application, and administration of the pulsed cells (i.e., the composition) to animals, resulted in *in vivo* stimulation of IFN-gamma by the 17-mer Cripto peptide (i.e there is antigen recall). The degree of stimulation was comparable with the response to Con A, the positive control, but substantially higher than the control where immune cells were not exposed to any antigens (indicated by a "-" under the histogram at the extreme right in each of the figures).

The antigen recall of the 17-mer Cripto peptide was also substantially greater than with VNTR, indicating a specific antigen selection and presentation by APCs, and leading to generation of a T cell immune response to the Cripto peptide *in vivo*.

In conclusion, the results show that the Cripto 17 mer peptide, a cancer antigen that is distinct from Muc1, can stimulate DCs *ex vivo* when conjugated with mannan, enabling the pulsed DCs to stimulate T cell immune response to the antigen *in vivo* following administration.

5. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and

that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: 20/10/04 By: G.A Pietersz
Geoffrey A. Pietersz